Kaleidoscope

Volume 9 Article 30

November 2011

Diurnal Variations of Genes Contributing to Sodium and Potassium Cardiac Currents

Parvathi Nataraj

Parvathi Nataraj

Diurnal Variations of Genes Contributing to Sodium and Potassium Cardiac Currents

The electrical activity of the heart is based on the differing ionic conductances across the cardiac myocyte membrane. In a cardiac action potential, the opening of voltage-gated sodium channels (Na^+ channels) leads to a large increase in Na+ conductance, which depolarizes the membrane. The repolarization of the membrane is due to the opening of voltage-gated potassium channels. Mutations in cardiac ion channels can lead to cardiac arrhythmias. Mutations in SCN5A, which codes for Nav 1.5, the alpha subunit of the cardiac sodium channel have been linked to Brugada syndrome and Long QT type 3 (LQT3) syndrome, both of which can lead to ventricular arrhythmias and sudden cardiac death. It has been shown that patients with Brugada syndrome and LQT3 syndrome are more likely to experience cardiac arrhythmias in the night than during the day. The increased risk of arrhythmias occurring at night in Brugada or LQT3 patients suggests that there is a diurnal variation associated with these disorders.

The purpose of this study is to determine if four genes, which regulate the cardiac sodium and potassium currents (*SCN5A, GPD1L, SCN4B,* and *KCNJ2*) are regulated diurnally. (*GPD1L* and *SCN4B* both aid in the regulation of the cardiac sodium current while *KCNJ2* regulates the potassium current.)

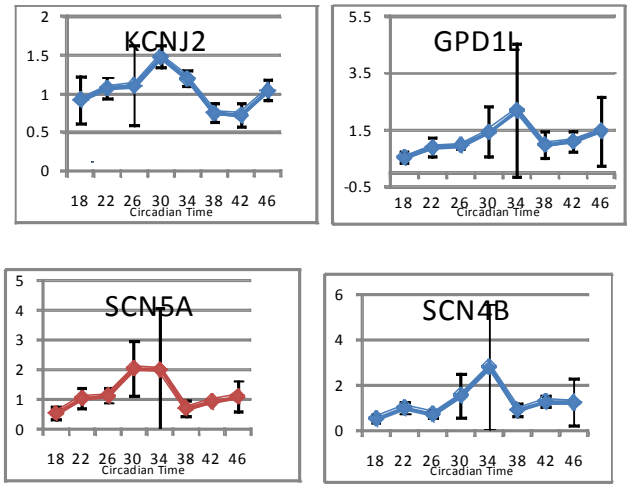

Figure 1 Expression levels of the genes KCNJ2, GPD1L, SCN5A, and SCN4B from Circadian time 18 to 46. (The vertical axes represent the concentrations of DNA after normalization to GAPDH.)

In order to test the hypothesis, intracellular mRNA and protein levels must be measured at various time points. Murine cardiac tissue isolated from the apex of the heart was used for the experiments. The mice used for this study were kept in a 12 hour light/dark cycle for at least two weeks. After this time period, the mice were kept in constant darkness while collections of cardiac tissue were done every four hours from circadian time 18 to 50 (9 collections in total).

In order to determine the transcription of the genes at different times during the day, real time PCR was used. Real time PCR is a method used to quantitatively amplify DNA. This technique allows one to compare the expression levels of different genes. Total RNA was extracted from the cardiac tissue and reverse transcriptase was used to make cDNA (copy DNA), which was quantified using real time PCR. Primers for the genes *SCN5A, SCN4B, GPD1L,* and *KCNJ2* were used. All data for the expression levels of the genes were gathered in triplicate, and for each gene, samples from three hearts were used. The expression of the genes being tested was normalized to the expression of Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), since *GAPDH* is not expressed diurnally. Data sets are shown in Figure 1.

The results of the experiments so far seem quite promising. As seen above, *SCN5A*, which is thought to be related to Brugada syndrome and codes for the protein Nav 1.5, may be transcribed in a diurnally variating pattern as well as *KCNJ2*, which codes for the protein Kir 2.1. Nav 1.5 is the alpha subunit of the cardiac sodium channel in the heart. Kir 2.1 is a cardiac inward rectifier potassium channel.

The last step of the project will deal with the quantification of the protein products of the genes *SCN5A* and *KCNJ2* (Nav 1.5 and Kir 2.1) by Western blotting. The protein levels will then be compared to a control protein which remains at a stable concentration. It is expected that the proteins Nav 1.5 and Kir 2.1 will be produced in a diurnal pattern.

Acknowledgments

I am grateful to Dr. Brian Delisle and Dr. Elizabeth Schroder for their invaluable assistance and advice.